A perspective on paper-based microfluidics: Current status and future trends

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"Paper-based microfluidics" or "lab on paper," as a burgeoning research field with its beginning in 2007, provides a novel system for fluid handling and fluid analysis for a variety of applications including health diagnostics, environmental monitoring as well as food quality testing. The reasons why paper becomes an attractive substrate for making microfluidic systems include: (1) it is a ubiquitous and extremely cheap cellulosic material; (2) it is compatible with many chemical/biochemical/medical applications; and (3) it transports liquids using capillary forces without the assistance of external forces. By building microfluidic channels on paper, liquid flow is confined within the channels, and therefore, liquid flow can be guided in a controlled manner. A variety of 2D and even 3D microfluidic channels have been created on paper, which are able to transport liquids in the predesigned pathways on paper. At the current stage of its development, paper-based microfluidic system is claimed to be low-cost, easy-to-use, disposable, and equipment-free, and therefore, is a rising technology particularly relevant to improving the healthcare and disease screening in the developing world, especially for those areas with no- or low-infrastructure and limited trained medical and health professionals. The research in paper-based microfluidics is experiencing a period of explosion; most published works have focused on: (1) inventing low-cost and simple fabrication techniques for paper-based microfluidic devices; and (2) exploring new applications of paper-based microfluidics by incorporating efficient detection methods. This paper aims to review both the fabrication techniques and applications of paper-based microfluidics reported to date. This paper also attempts to convey to the readers, from the authors' point of view the current limitations of paper-based microfluidics which require further research, and a few perspective directions this new analytical system may take in its development.
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THE FABRICATION OF PAPER-BASED MICROFLUIDIC DEVICES

The invention of the paper-based microfluidics, which has been attributed to the Whitesides Group of Harvard University, actually has a longer history; the earlier work reported by Müller *et al*. may be the origin of this technology (Figure 1). In 1949, Müller and a co-worker conducted a study on the preferential elution of a mixture of pigments within the channel on paper.[1] They patterned filter paper by impregnating a paraffin barrier on the paper and observed that the confined channel sped up the sample diffusion process and reduced sample consumption. This paraffin-patterned paper could therefore be regarded as the rudiment of the paper-based microfluidics developed over recent years.

Overall, there are ten techniques reported in the literature for fabricating paper-based microfluidic devices: (1) photolithography,[2–4] (2) plotting with an analogue plotter,[5] (3) ink jet etching,[6,7] (4) plasma treatment,[8,9] (5) paper cutting,[10,11] (6) wax printing,[12–14] (7) ink jet

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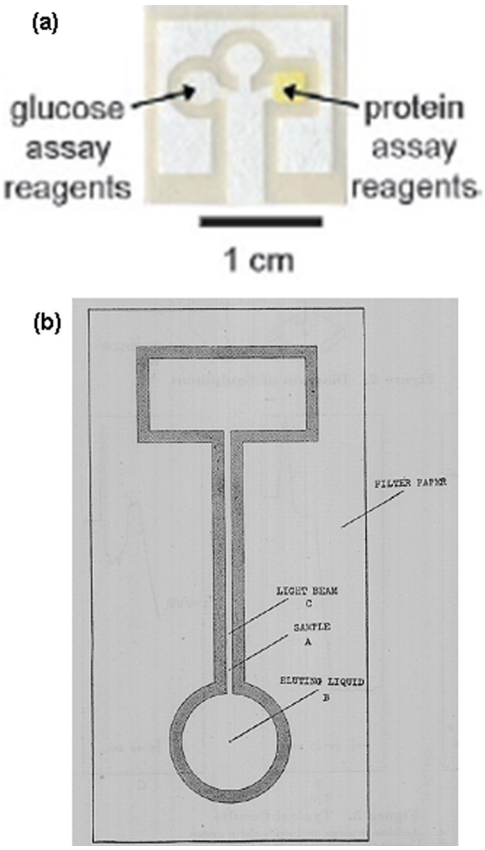

FIG. 1. Paper-based microfluidic channels created using (a) photolithography technique by Martinez *et al*. in 2007 and (b) paraffin impregnation method by Müller *et al*. in 1949 (adapted with permission from Müller and Clegg, Analytical Chemistry **21**(9), 1123 (1949). Copyright © 1949 American Chemical Society; adapted with permission from Martinez *et al*., Angewandte Chemie, International Edition **46**(8), 1318 (2007). Copyright © 2007 John Wiley and Sons.).

printing,[9,15,16] (8) flexography printing,[17] (9) screen printing,[18] and (10) laser treatment.[19] The fundamental principle underlying these fabrication techniques is to pattern hydrophilic-hydrophobic contrast on a sheet of paper in order to create micron-scale (i.e., hundreds to thousands of micrometers) capillary channels on paper. The cutting technique is an exception to this methodology; it first shapes paper pattern by cutting it with a computer controlled plotter cutter and then encases the shaped paper with sticky tape, which serves as a backing to create paper-based microfluidic devices. Since this technique does not rely on the hydrophilic-hydrophobic contrast, it will not be included in the following discussion.

Table I lists the different patterning agents (i.e., hydrophobic agents), patterning principles, and approaches for each fabrication technique. A variety of hydrophobic substances have been utilized to define hydrophilic micro-channels on paper, from the relatively expensive agents such as photoresist SU-8 (~$0.1 for patterning filter paper of 100 cm^2),[20] the less expensive agents such as wax (~$0.01 for patterning filter paper of 100 cm^2)[13] to the extremely cheap agents such as alkyl ketene dimer (AKD, ~$0.00001 for patterning filter paper of 100 cm^2).[9]

Based on the binding states of hydrophobic agents to paper, the paper patterning principles of these techniques can be divided into three categories: physical blocking of the pores in paper (using agents such as photoresist and polydimethylsiloxane (PDMS)), physical deposition of a hydrophobizing reagent (e.g., polystyrene or wax) on the cellulose fibre surfaces, and chemical modification of fibre surfaces (using cellulose reactive agents such as AKD which is a commodity grade of paper sizing/hydrophobizing agent used in the papermaking industry). Physical pore blocking and physical fibre surface modification do not involve any chemical reactions between the hydrophobic agents and the cellulose fibres; these agents are physically impregnated into

TABLE I. Comparison of the ten published techniques for patterning hydrophilic-hydrophobic contrast on paper to create paper-based microfluidics.

Fabrication techniques	Patterning agents	Patterning principles	Patterning approaches
Photolithography[2–4]	Photoresist (e.g., SU-8)	Physical blocking of pores in paper	Entire hydrophobization followed by selective dehydrophobization
Plotting[5]	PDMS	Physical blocking of pores in paper	Selective hydrophobization
Ink jet etching[6,7]	Polystyrene	Physical deposition of reagent on fibre surface	Entire hydrophobization followed by selective dehydrophobization
Plasma treatment[8,9]	AKD	Chemical modification of fibre surface	Entire hydrophobization followed by selective dehydrophobization
Wax printing[12–14]	Wax	Physical deposition of reagent on fibre surface	Selective hydrophobization
Ink jet printing[9,15,16]	AKD	Chemical modification of fibre surface	Selective hydrophobization
Flexography printing[17]	Polystyrene	Physical deposition of reagent on fibre surface	Selective hydrophobization
Screen printing[18]	Wax	Physical deposition of reagent on fibre surface	Selective hydrophobization
Laser treatment[19]	Depend on paper types (e.g., silicone for parchment paper, wax for wax paper)	Physical blocking of pores in paper	Entire hydrophobization (during papermaking process) followed by selective dehydrophobization

paper pores or deposited onto the fibre surface. The presence of these agents changes the liquid wetting properties of the paper, making the formation of hydrophilic-hydrophobic patterns in paper possible. Chemical modification of fibre surfaces is achieved by applying cellulose reactive agents to paper; these agents typically react with the −OH groups of cellulose, imparting hydrophobicity to cellulose fibres.[9] Generally, paper hydrophobicity caused by chemical modification cannot be removed by organic solvent extraction,[21] whereas paper hydrophobicity caused by physical deposition can be largely removed by organic solvent washing, making it possible to use organic solvent etching methods to fabricate paper-based microfluidic devices.[6,7]

The patterning approaches for each technique are classified into: (i) selective hydrophobization (one-step fabrication) and (ii) entire hydrophobization followed by selective dehydrophobization (two-step fabrication). The former approach is to directly deposit hydrophobic agents to the selected areas of a sheet of paper to hydrophobize these areas; areas not receiving patterning agents remain hydrophilic. The prevailing techniques for achieving this contrast include wax printing[12,13] and ink jet printing.[15,16] The latter approach involves first making the entire paper sheet hydrophobic and then etching selected areas of the hydrophobic paper to form hydrophilic regions using techniques such as photolithography,[2,3] ink jet etching,[6,7] and patterned plasma oxidation.[8,9]

The advantages and limitations of each fabrication technique are listed in Table II. To choose the proper technique, researchers and manufacturers should consider a range of factors including equipment availability, material costs, fabrication process simplicity, and the intended applications of paper-based microfluidic devices. At present, AKD ink jet printing and wax printing might be the most promising techniques due to the low cost of patterning agents and easy, rapid fabrication process; both techniques can produce multiple devices or multizones on a piece of A4-sized paper (Figure 2) within 10 min with a single print-and-heat cycle. Both techniques have the potential to be upgraded to pilot or even larger scale processes by which paper could first go through an ink jet printer (or wax printer), then through an oven for heat treatment to form hydrophilic-hydrophobic contrast, and finally through other ink jet printers which can print detection reagents required for assays or other applications (Figure 2(a)).

TABLE II. Description of the main advantages and drawbacks for different fabrication techniques of paper-based microfluidic devices.

Fabrication techniques	Advantages	Drawbacks
Photolithography	High resolution of microfluidic channels (channel width is as narrow as 200 μm; the barrier is sharp)	Requires expensive equipment; requires an extra washing step to remove un-crosslinked polymer; devices are vulnerable to bending
Plotting	Patterning agent (PDMS) is cheap; devices are flexible	Deteriorated barrier definition; cannot be readily applied to high throughput production
Ink jet etching	Requires only a single printing apparatus to create microfluidic channels by etching and to print bio/chemical sensing reagents	Creation of microfluidic channels requires 10 times of printing; the printing apparatus must be customized; not suitable for mass fabrication
Plasma treatment	Uses very cheap patterning agent (AKD); dramatically reduces the material cost	Requires different masks for creating different microfluidic patterns on paper
Wax printing	Produces massive devices with simple and fast (5-10 min) fabrication process	Requires expensive wax printers; requires an extra heating step after wax deposition
Ink jet printing	Uses very cheap AKD; produces massive devices fast (<10 min) and simply; requires only a desktop printer to produce devices and to print sensing reagents	Requires an extra heating step after AKD deposition; requires modified ink jet printers
Flexography printing	Allows direct roll-to-roll production in existing printing houses; avoids the heat treatment of printed patterns	Requires two prints of polystyrene solution; requires different printing plates; print quality relies on the smoothness of paper surface
Screen printing	Produces devices with simple process	Low resolution of microfluidic channels (rough barrier); requires different printing screens for creating different patterns
Laser treatment	High resolution (minimum pattern size of about 62 μm)	Microfluidic channels do not allow lateral flow of fluids; requires extra coating for liquid flow

THE APPLICATION OF PAPER-BASED MICROFLUIDIC DEVICES

The main application of paper-based microfluidic devices is to provide a low-cost, easy-to-use, and portable analytical platform for assays, either multi-analyte or semi-quantitative (even quantitative), in order to provide people living in the developing world with affordable disease diagnosis and environmental monitoring. In terms of this application, paper-based microfluidic devices can be divided into two types: (1) On-demand devices, which are blank microfluidic platforms without pre-deposited indication reagents. Depending on the samples to be tested, the detection reagents are chosen and introduced into the devices by users prior to the test, either before or after the adding of test samples (Figure 3). (2) Ready-to-use devices, which are designed as complete sensors by integrating indication reagents into the detection zones of the devices. Based on the particular detection chemistry incorporated, this type of device is used to detect specific analytes in test samples (Figure 4).

Using the on-demand device, the simultaneous detection of the enzyme activity of different samples was implemented in a same device;[8] a single device was also used to semi-quantitatively or quantitatively measure the concentration of an analyte by concurrently detecting the unknown sample solutions and the standard solutions in the different detection zones of the device, one example is the quantification of nitrite in an unknown sample.[22] Most of the published works to date have been focusing on the applications of the ready-to-use device. These applications include: (1) the simultaneous detection of multiple analytes in a sample solution, a well-studied case is to use the glucose/protein paper-based sensor for the

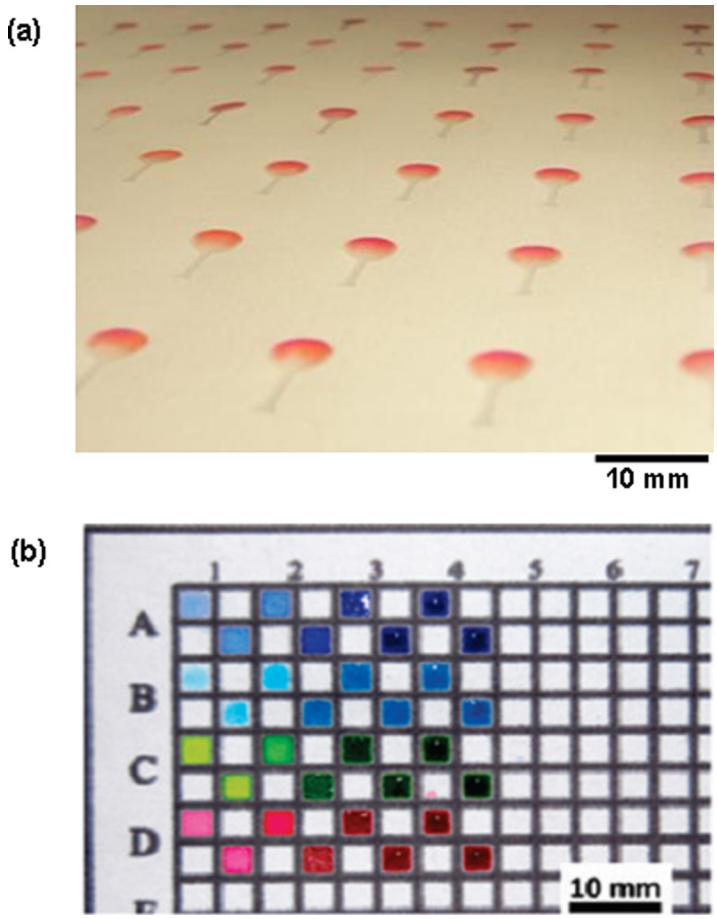

FIG. 2. Examples of multiple paper-based microfluidic devices and multiple paper-based microzones fabricated on an A4 size filter paper. (a) An example of the AKD ink jet-printed multiple paperfluidic devices. The colorless indicator for detecting NO_2^- was printed into each circular detection zone. NO_2^- sample solution (5 μl, 5 mM) was introduced into the channel of each device. As the sample solution reached the detection zone of each device, reproducible color changes were seen; (b) an example of a wax-printed 384-zone paper plate after the application of several dyes (adapted with permission from Carrilho *et al*., Analytical Chemistry **81**(16), 7091 (2009). Copyright © 2009 American Chemical Society; adapted with permission from Li *et al*., Colloids and Surfaces, B: Biointerfaces **76**(2), 564 (2010). Copyright © 2010 Elsevier.).

simultaneous determination of glucose and protein in a urine sample;[2,23] and (2) the semi-quantitative analysis of multiple analytes by detecting the unknown sample and the standard solutions using a set of devices, for instance, the quantification of glucose, lactate, and uric acid in a urine sample.[24] Table III lists all analytes studied to date for different applications of paper-based microfluidic devices. In addition, Table III also outlines the analytical applications of paper-based microarray plates. Such plates comprise multiple hydrophilic microzones on a sheet of paper[25,26] and can be fabricated by patterning paper using the aforementioned techniques for producing paper-based microfluidic devices. Paper-based microplates have been proposed as a potential substitute of the traditional microtiter plate for enzyme-linked immunosorbent assays (ELISA).[27]

In general, four detection methods have been reported for the detection of analytes in paper-based microfluidics: colorimetric detection, electrochemical (EC) detection, chemiluminescence (CL) detection, and electrochemiluminescence (ECL) detection (Table III). Colorimetric detection chemistries are typically related to enzymatic or chemical color-change reactions. In most cases, the analysis of results can be visually assessed by the unaided eye,[28] which is adequate when a yes/no answer or a semi-quantitative detection is sufficient for diagnosis.[29] EC detection has higher sensitivity, enabling the detection and quantification of analytes even in the nM range.

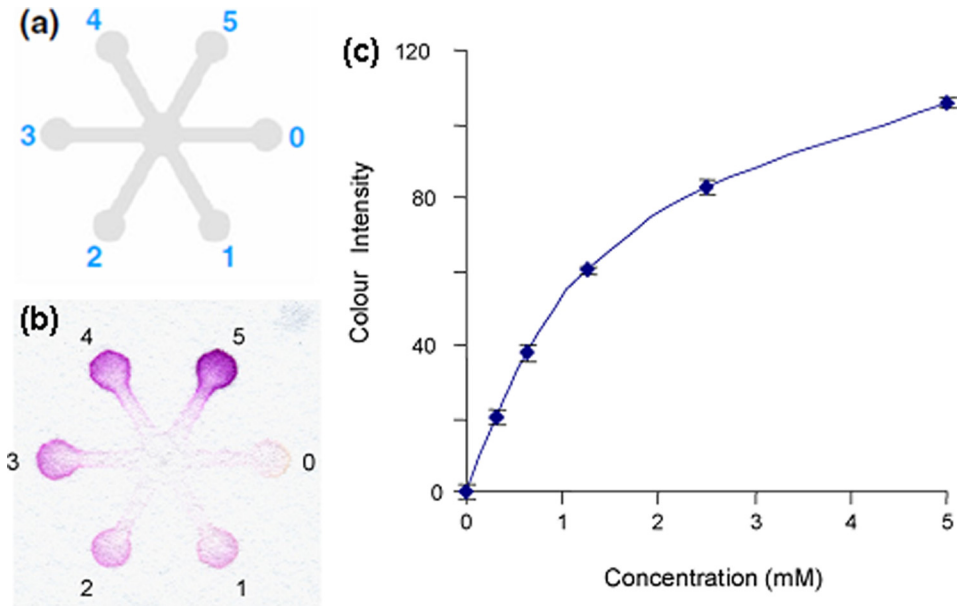

FIG. 3. On-demand paper-based microfluidic device for semi-quantitative assay. (a) The assay design: NO_2^- standard solutions (0.5 μl) of different concentration (0, 312, 625, 1250, 2500, and 5000 μM) were deposited in sequence into detection zone 0 to zone 5 of a blank device (gray region represents the hydrophilic area of the device); (b) NO_2^- indicator solution was added into the device from central inlet zone and caused color formation of different density in different detection zones. The device was scanned using a desktop scanner; (c) calibration curve was generated by measuring color density of each detection zone using Adobe Photoshop. Error bars were obtained from six repeated measurements (adapted with permission from Li *et al.*, Cellulose **17**(3), 649 (2010). Copyright © 2010 Springer Science+Business Media B.V.; adapted with permission from Li *et al.*, Colloids and Surfaces, B: Biointerfaces **76**(2), 564 (2010). Copyright © 2010 Elsevier.).

Besides, electrochemistry, unlike colorimetry, is insensitive to local lighting conditions, therefore, is less prone to interference from certain types of contaminants (suspended solids, colored materials) present in the samples.[30] Most studies reported to date have been focusing on exploiting the colorimetric detection (Figure 5(a))[2–12,15,17,18,22,24,27,31–37] and the EC detection (Figure 5(b))[18,30,37–40] in paper-based microfluidics. CL and ECL are the most common optical detection methods in microfluidics. They are performed in the dark and are therefore independent of ambient light. However, they have not been widely used in paper-based microfluidics; only a few

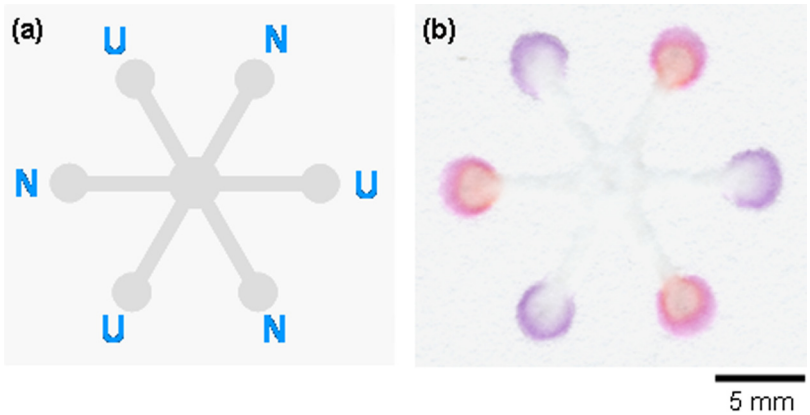

FIG. 4. Ready-to-use paper-based microfluidic device for multi-analyte detection. (a) The assay design: the device was incorporated with NO_2^- indicator (in detection zones N) and uric acid (UA) indicator (in detection zones U) after being fabricated (gray region represents the hydrophilic area of the device); (b) a mixed sample solution containing NO_2^- and UA was introduced from central inlet zone; it penetrated into each detection zone and triggered different color changes (pink for NO_2^- and purple for UA) (adapted with permission from Li *et al.*, Cellulose **17**(3), 649 (2010). Copyright © 2010 Springer Science+Business Media B.V.).

TABLE III. Summary of the reported detection methods, example analytes and applications for both paper-based microfluidic devices and paper-based microarray plates.

	Detection methods	Example analytes	Applications
Paper-based microfluidic devices	Colorimetric detection	Glucose,[2–6,10,12,17,24,31,33,34] protein (e.g., bovine serum albumin),[2,3,5,6,10–12,31,32,34] nitrite,[4,9,15,22,31] uric acid,[9,22,24] ketones,[4,31] lactate,[24] *p*H,[6,7] human IgG,[7] total iron,[18] pathogenic bacteria (e.g., *Pseudomonas aeruginosa*, *Staphylococcus aureus*),[36] ABO antigens[35]	Health diagnostics (e.g., urinalysis, saliva analysis, sputum analysis, pregnancy test, blood typing)
		Alkaline phosphatase[8,15]	Biochemical analysis (e.g., enzyme activity)
		Fe (III)[37]	Environment monitoring
	Electrochemical detection	Glucose,[18,30,38,39] cholesterol,[30] lactate,[30,39] Ascorbic acid (AA),[40] uric acid (UA),[39,40] total iron[18]	Health diagnostics
		Pb(II),[38] Au(III)[37]	Environment monitoring
		Ethanol[30]	Food quality control
	Chemiluminescence detection	Glucose,[41] uric acid[41]	Health diagnostics
	Electrochemiluminescence detection	Nicotinamide adenine dinucleotide (NADH)[16]	Biomedical analysis
Paper-based microarray plates	Colorimetric detection	Rabbit IgG,[27] HIV-1 antigen (gp41)	Biochemical analysis (e.g., ELISA)
	Chemiluminescence detection	Iron in the hemoglobin[19]	Forensic (e.g., detection of blood)

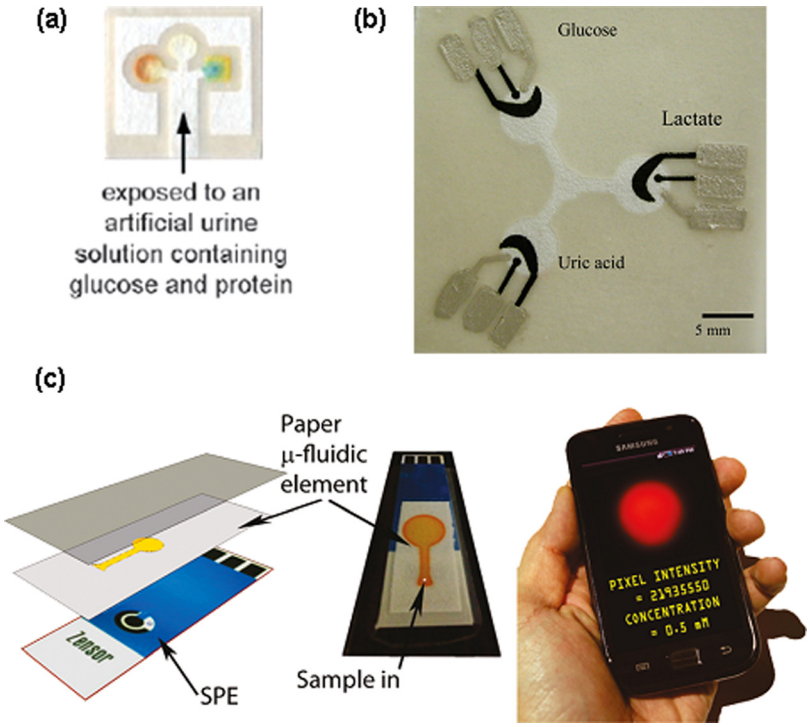

FIG. 5. Examples of three detection methods for bioassays on paper-based microfluidic devices. (a) Colorimetric detection for simultaneously detecting glucose and protein in an artificial urine sample; (b) EC detection on a three-electrode paper-fluidic device. The hydrophilic area at the center of the device wicked sample into the three separate test zones. The silver electrodes and contact pads were made from Ag/AgCl paste with the black electrode portions being the Prussian Blue-modified carbon electrodes; (c) ECL detection of a sample solution (2-(dibutylamino)-ethanol (DBAE)). The device was filled with a 10 mM $Ru(bpy)_3^{2+}$ solution before drying, and was then aligned and fixed onto the face of the screen-printed electrode (SPE) by laminating with transparent plastic. A drop of sample was introduced through a small aperture in the plastic at the base of the channel. After the detection zone being fully wetted, a potential of 1.25 V was applied and the resulting emission was captured and analyzed (adapted with permission from Martinez *et al*., Angewandte Chemie, International Edition **46**(8), 1318 (2007). Copyright © 2007 John Wiley and Sons; adapted with permission from Delaney *et al*., Analytical Chemistry **83**(4), 1300 (2011). Copyright © 2011 American Chemical Society; adapted with permission from Dungchai *et al*., Analytical Chemistry **81**(14), 5821 (2009). Copyright © 2009 American Chemical Society.).

studies investigated using these two detection methods for detecting analytes in paper-based microfluidic devices or paper-based microarray plates.[16,19,41] Figure 5(c) shows an approach of conducting ECL detection on paper-based microfluidic devices.

Although paper-based microfluidic devices provide a low-cost and simple platform for performing multi-analyte detection and semi-quantitative measurement, more complex devices are required where there are multiple steps involved in the analysis/diagnosis, such as the premixing of samples before the final reaction. Under such circumstances, functional elements could be incorporated into the devices for controlling the movement of fluids within paper-based microchannels and for expanding the functions of the devices. For example, Li *et al*. designed a paper-based separator for filtering samples and a paper-based reactor for multi-step reactions (Figure 6);[8] Whitesides group reported the fabrication of 3D paper-based microfluidic devices, which can distribute fluids both vertically and laterally, enabling liquid transport from a single inlet to numerous detection zones (Figure 7(a)).[3] This group also designed a programmable device with "on" buttons for connecting and disconnecting the fluid flow between paper-based channels;[31] Liu and Crooks reported using the principles of origami for fabricating 3D paper-based microfluidic devices (Figure 7(b)).[42] The research group led by Yager has executed comprehensive studies on the characterization of fluid flow in paper-based microfluidic channels and on controlled liquid sample transport;[43–46] Noh *et al*. described one method to control when and how quickly a fluid is distributed into detection zones by integrating paraffin wax

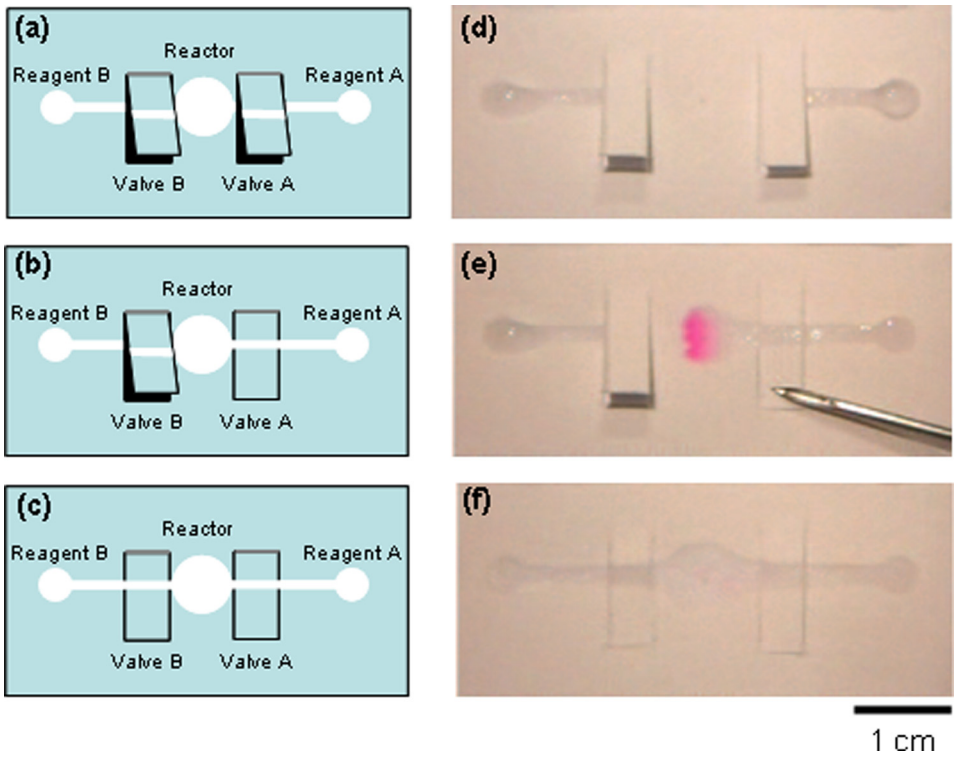

FIG. 6. A paper-based microfluidic reactor for multi-step reactions. (a)-(c) The design of the paper-based reactor which consists of two sample dosing sites, two valves, and one central reaction site; (d)-(f) the paper-based reactor was tested using an acid–base neutralization reaction ((d) Phenolphthalein indicator solution was deposited onto the central reaction zone. NaOH and HCl solutions were added into reagent zones A and B, respectively; (e) NaOH solution was introduced into the reaction zone via valve A to trigger color change; (f) HCl solution was introduced later into the reaction zone via valve B to neutralize NaOH in the reaction zone) (adapted with permission from Li *et al*., Cellulose **17**(3), 649 (2010). Copyright © 2010 Springer Science+Business Media B.V.).

into μPADs as fluidic timers;[33,47] In a more recent study, Yang *et al*. designed a paper-based microfluidic device for separating blood plasma from small samples of whole blood.[48]

In addition to the applications in analyte detection, paper-based microfluidic devices and microarray plates have also been used for other applications including: (1) to be used as a template for fabricating thin materials such as films of ionotropic hydrogels which are used in drug delivery, for encapsulation of cells and as sorbents for toxic metals in wound dressings;[49–52] (2) to be used as a stamp for the contact printing of biochemicals such as reagents, antigens, proteins, and DNA onto planar substrates;[53] (3) to be used as a flexible substrate for rapidly prototyping PDMS microdevices, which could simplify the operation procedures and reduce the fabrication cost of the devices;[54] and (4) to be used as a platform for 3D cell culture and 3D cell-based analysis.[55,56]

THE CURRENT LIMITATION OF PAPER-BASED MICROFLUIDIC DEVICES

The outstanding features of paper-based microfluidic devices were extensively investigated in numerous studies and can be found in review articles published elsewhere.[57–59] It needs, however, to be pointed out that, currently, paper-based microfluidic devices do have limitations, which are related to the material properties of paper, the fabrication techniques of the devices or the detection methods incorporated to the devices. Specifically, these limitations include the following:

(1) The sample retention (i.e., the ineffective sample consumption) within paperfluidic channels and the sample evaporation during transport result in the low efficiency of sample delivery within the device. The volume that reaches the detection zones verses the total volume within

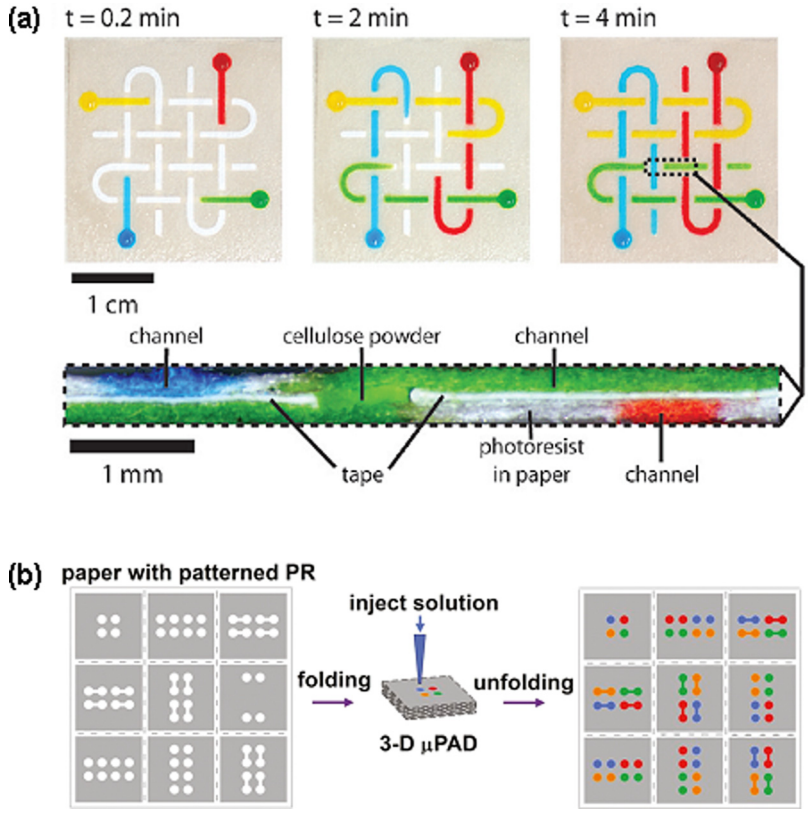

FIG. 7. Two types of 3D paper-based microfluidic devices. (a) A 3D device was made by stacking two layers of patterned paper and one layer of double-sided adhesive tape. The holes in the tape were filled with cellulose powder to allow fluids to penetrate between adjacent layers of paper; (b) the scheme of a 3D device assembled using the principles of origami (PR, photoresist; μPAD, microfluidic paper-based analytical device) (adapted with permission from Liu and Crooks, Journal of the American Chemical Society **133**(44), 17564 (2011). Copyright © 2011 American Chemical Society; adapted with permission from Martinez *et al*., Analytical Chemistry **82**(1), 3 (2009). Copyright © 2009 American Chemical Society.).

the device is usually less than 50%. In situations where the sample quantity is tiny or the sample is costly, low-volume sensors with more efficient sample delivery are desirable.

(2) Some hydrophobic agents for patterning devices cannot build hydrophobic barriers strong enough to withstand samples of low surface tension; for example, the hydrophobic areas of a paper-based microfluidic device which is fabricated with wax or AKD do not allow the liquid penetration only when the surface tension of the liquid is higher than a critical value. When the surface tension of a liquid is lower than that value (e.g., biological samples with surfactant), liquid can penetrate not only within the hydrophilic channels but also in the hydrophobic areas of the device, rendering the device ineffective for guided liquid transport. This is because wax and AKD hydrophobize paper by lowering the surface free energy of the paper, not blocking the pores in paper.

(3) The limit of the detection (LOD) is usually high for the traditional colorimetric method integrated into the devices, making the current paper-based microfluidic devices insufficient for the analysis of samples of very low concentration. For example, the permitted maximum level of contaminants in drinking water or food is typically set in the ppb or even ppt range, which may be undetectable by the conventional colorimetry.

These current limitations of paper-based microfluidic devices will require further research to overcome. To circumvent the above-mentioned limitations, a number of studies have been conducted using alternative cheap materials, fabrication techniques, and detection methods for creating new types of low-cost microfluidic systems. Tian *et al*. designed the V-groove microfluidic devices by using non-porous capillary channels to transport samples and paper-like

porous zones to detect samples.[60] Compared to the porous paper channel, the non-porous V-groove channel on polymer film can significantly reduce sample retention and cause less chromatographic separation of the analytes in samples, thus increasing the sample delivery efficiency. Li *et al*. invented the low-cost thread-based microfluidic system, with which the 3D microfluidic devices were created by sewing threads through a supporting substrate such as a polymer film; these devices have higher wet strength compared with paper-based microfluidic devices,[61] and fluid mixing can be easily achieved by twining threads together as a mixing microfluidic channel.[62] Reches *et al*. also reported using thread as an alternative material for fabricating low-cost microfluidic systems.[63] Several studies on bioactive paper have proposed a few promising ideas to enhance the sensitivity and selectivity of the colorimetric detection for paper-based microfluidic devices. One proposal is combining assay reagents with sol-gel materials to immobilize reagents on paper.[64–66] In this way, Hossain *et al*. developed a paper-based sensor which can detect organophosphate pesticides of nanomolar quantities in milk or lettuce by color changing.[65] Another proposal is using gold nanoparticle (AuNP) based colorimetric detection which can dramatically improve both the sensitivity and the specificity of detection.[67] Zhao *et al*. applied a AuNP colorimetric probe to paper-based assays for detecting Deoxyribonuclease I and adenosine.[68]

THE FUTURE PERSPECTIVES

Research on paper-based microfluidic devices is still at an early stage; significant research efforts will be needed in this field to nurture it into a more matured platform technology in diagnostic, point-of-care (POC), and environmental monitoring applications. Further exploratory studies will be seen which discover new concepts and capabilities of this technology. Whilst any individual researcher in this field may come up with a list of potential future directions from their point of view, here, we hope to convey to readers a few of the perspective directions which we think are relevant and attractive in this field.

Although new fabrication methods of paper-based microfluidic devices will continually be reported in future, the practicality of the existing and future methods will be judged by the POC and diagnostic market in terms of the material and fabrication costs, their potentials for mass productions, their reliance upon any other equipment in order to function, their reliability in providing easy-to-interpret assay results, and their compatibility to telemedicine, particularly with mobile phone transmission or interpretation of test results.[16,23] Whilst a major advantage of paper-based microfluidics is that they can function in an equipment-free fashion, simple, and portable result-interpretation devices have been reported.[32] However, paper-based microfluidic devices that rely on complicated instrumentation for result interpretation may only have value for laboratory uses.

Laboratories with experience in papermaking, converting, and printing technologies may, in future, play increasing roles in tailoring novel, functionalized and bioactive paper substrates, and mass fabrication processes to speed up the commercialization process of paper-based microfluidic diagnostics for real POC and disease screening applications. Their expertise in controlling paper sheet structures,[17] incorporating new (e.g., nanocellulose fibres), and biofunctional materials into sheets using polyelectrolytes,[69] and printing technology[17] will significantly benefit the future development of paper-based microfluidic technology. To date, most paper-based microfluidic devices are made using filter papers; in future other grades of papers that have certain unique properties can be used for making certain paper-based devices for specific applications. These new devices will have properties that cannot be provided by filter papers.

Further research on mechanistic understanding of capillary wicking within paper sheets will be necessary for gaining more precise control of lateral flow in paper.[43,46,70] Paper surface energy[33] and structure[17] may be the most relevant parameters which attract investigation. Also, vertical flow in paper (i.e., liquid flow through the paper thickness through a defined area in a controllable rate) may need investigation. This is because diagnostic devices based on vertical sample flow are possible; another reason is that 3D paper-based microfluidics will require the control of not only liquid lateral flow but also vertical flow.

Novel signal display methods may attract investigation. One of the possibilities for paper-based microfluidics is to display the testing results in text since paper is inherently suited to displaying text. Text-reporting is much more user-friendly to untrained users of paperfluidic devices; it is, therefore, a useful field of research to explore. Although printing and displaying text using enzyme and other simple colorimetric chemistries on paper microfluidic devices have been shown to be possible,[71,72] studies of using text to report diagnostic results on paper are extremely rare.[73]

The value of a new technique or a new product could only be embodied after it has been commercialized and accepted by the potential users. Similarly, paper-based microfluidics, as a new platform or system for liquid manipulation and sample detection, will also need time to attest its real value to society.

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